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The antidepressant-like effect of *Hedyosmum brasiliense* and its sesquiterpene lactone, podoandin in mice: Evidence for the involvement of adrenergic, dopaminergic and serotonergic systems

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ABSTRACT

We have recently shown that the ethanol extract of the leaves of *Hedyosmum brasiliense* exhibits an antidepressant-like effect in the tail suspension and forced swimming tests in mice. The present study investigates the mechanisms involved in the antidepressant-like effect of *H. brasiliense* extract, together with the antidepressant potential of podoandin, an isolated sesquiterpenoid. *H. brasiliense* (50 mg/kg, i.p.) and podoandin (10 mg/kg, i.p.) decreased the immobility time in the forced swimming test, without any accompanying changes in ambulation in the open-field test. The anti-immobility effect of the *H. brasiliense* extract was prevented by pre-treating the mice with ondansetron, NAN 190, pindolol, prazosin, yohimbine, haloperidol, SCH23390, and sulpiride. On the other hand, pre-treating the mice with: *p*-chlorophenylalanine (4 consecutive days), ketanserin, naloxone, naltrindole, bicuculline, phaclofen, or L-arginine did not block the antidepressant-like effect of *H. brasiliense*. In addition, pre-treatment of the animals with methylene blue, NG-nitro-L-arginine or 7-nitroindazole, at subeffective doses, did not cause a synergistic effect with *H. brasiliense* extract at an effective dose in the forced swimming test. The anti-immobility effect of podoandin was also prevented by pre-treating the mice with NAN-190, ondansetron, prazosin, yohimbine, sulpiride and haloperidol. The results indicate that the antidepressant-like effect of *H. brasiliense* (and podoandin) is dependent on the serotonergic, noradrenergic and dopaminergic systems, but not on the GABAergic, opioid and oxidonitrergic systems.

1. Introduction

After about fifty years of the discovery of the first antidepressant (Castrén, 2005), depression, which is a chronic, relapsing and potentially fatal disease, appears to be increasing. It currently affects about 20% of the population worldwide, and is considered one of the ten leading causes of morbidity and mortality. It is estimated that by the year 2020, depression will be the 2nd largest contributor to the global burden of disease (WHO, 2011a,b). Numerous antidepressant compounds are now available, which presumably act via different mechanisms, including the serotonergic, noradrenergic and/or dopaminergic systems. The therapies available for treating this disease, including tricyclic antidepressants, monoamine oxidase inhibitors, and serotonin reuptake inhibitors, are often associated with various undesirable side effects, and their efficacy benefits only certain parts of the world's population (Berton and Nestler, 2006). Moreover, most therapies require several weeks of treatment before any improvement in the signs and symptoms is observed. Despite the advances in drug discovery, and the therapeutic options available, there are still many shortcomings that need to be remedied. Medical plant therapies may be effective alternatives for the treatment of depression. Research in this area has progressed significantly in the past decade (Machado et al., 2007; WHO, 2011a,b) and this has led to the search for new alternatives for the treatment of depression (Buller and Legrand, 2001).

Hedyosmum brasiliense is an aromatic shrub, commonly known as Cidrão, which belongs to the largest genus of the Chloranthaceae family and is endemic to Brazil. The genus consists of 46 species found in tropical and subtropical regions of America (Souza and Lorenzi, 2005). Although widely used as a calmative, hypnotic, antidepressant, stomachic, and aphrodisiac, to treat migraine, and for diseases of the ovaries (Reitz, 1965), studies attempting to validate the pharmacological effects are limited. Only its antinociceptive effect (Trentin et al., 1999) has been determined previously to our series of investigations, which includes analysis of its essential oil composition and antimicrobial activity (Kirchner et al., 2010), the neurochemical properties of the ethanol extract, fractions and the antidepressant effect of isolated sesquiterpene lactones (onoseriolide or 13-hydroxy-8,9-dehydroshizukanolide and podoandin, **1**) which were assessed by

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the open field, elevated-plus-maze, pentobarbital induced sleeping time, forced swimming, strychnine and pentylenetetrazole-induced seizure and inhibitory avoidance tests (Tolardo et al., 2010). In the latter, an antidepressant effect was only observed for the sesquiterpenolide podoandin, which exhibited a significant reduction in immobility time. Continuing this research, this paper proposes a mechanistic investigation of the antidepressant effect of the extract, as well as that of podoandin which is an isolated sesquiterpene lactone of the guaianolide type. Some recently investigated guaianolides showed antiprotozoal (Maas et al., 2011; Sun et al., 2010), analgesic and sedative (Wesolowska et al., 2006) and cytotoxic (Bruno et al., 2005) effects.

2. Materials and methods

2.1. Extract and compound isolation

Aerial parts of *H. brasiliense* Miq. Chloranthaceae were collected in October of 2009 in Antônio Carlos, Santa Catarina State, Brazil. Voucher specimens were analyzed by the botanist Dr. Ademir Reis, and deposited at the Lyman Bradford Smith Herbarium (UNIVALI, Itajaí — SC) (number 2031). Five kilograms of fresh leaves of *H. brasiliense* was blended with bi-distilled alcohol and macerated for 15 days. The extract obtained was then concentrated in a rotary evaporator. The recovered ethanol was used to re-macerate the plant, and the combined concentrated extracts were kept in a desiccator under vacuum to remove residual solvent, yielding 200 g of crude extract, of which an aliquot, named extract of *H. brasiliense*, was used for the pharmacological tests. To isolate the sesquiterpene lactone podoandin, the rest of the extract was then diluted in water and partitioned with solvents of increasing polarity, yielding the following fractions: hexane (16 g), dichloromethane (4 g), ethyl acetate (13 g) and a residual aqueous fraction, which was kept in the freezer. The hexane fraction was then subjected to *flash* column chromatography with silica gel CC (230–400 mesh) using *n*-hexane with increasing concentrations of CH_2Cl_2 (0–70%) and subsequently using CH_2Cl_2 with increasing concentrations of EtOAc (0–70%), (200 ml for each concentration), which yielded sub-fractions A to H. Re-crystallization of sub-fraction B led to the isolation of the guaianolide podoandin (Fig. 1, 300 mg) with a spectral purity grade identified by Nuclear Magnetic Resonance (NMR) spectra in comparison with previously published data (Blay et al., 2000).

2.2. Animals

Male Swiss mice at 8 weeks of age, weighing 23–30 g were maintained at constant room temperature (22–27 °C) with free access to water and food, under a 12:12 h light–dark cycle (lights on at 07:00 h). The mice were allowed to acclimatize to the holding room for 24 h before the behavioral procedure. All experiments were carried out between 13:00 and 17:00 h, and each animal was used only once (n = 8–10 animals per group). All the experiments were conducted blind in terms of the treatment condition of the animals. The USA National Institute of Health Guidelines for Animal Care and Use were followed, and the experiments were approved by the Animal Care and Ethical Committees of the University do Vale do Itajaí, under number 007/10. Each animal was used only once. All efforts were made to minimize animal suffering and reduce the number of animals used in the experiments.

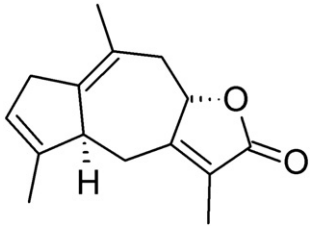

Fig. 1. Structure of podoandin (**1**), isolated from *H. brasiliense*.

2.3. Drugs and treatment

The following drugs were used: L-arginine, methylene Blue, 7-nitroindazole, NG-nitro-L-arginine, parachlorophenylalanine (PCPA), ketanserin, Nan 190 (1-(2-Methoxyphenyl)-4-[4-(2-phthalimido) butyl]piperazine hydrobromide), SCH 23390 (R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride), prazosin, phaclofen, bicuculline, yohimbine, haloperidol, pimozide, naloxone, naltrindole and pindolol, all provided by Sigma Chemicals (St. Louis, MO, USA). Ondansetron was provided by Cristália (Brazil).The extract of *H. brasiliense* and podoandin was dissolved in saline with 10% Tween 80, and all the other drugs were dissolved in isotonic saline solution (NaCl 0.9%) immediately before use. All the drugs, the podoandin and the *H. brasiliense* extract were administered by the intraperitoneal (i.p.) route, at a volume of 10 ml/kg body weight. The control animals received appropriate vehicle. Podoandin (Fig. 1), *H. brasiliense*, or vehicle was administered by the intraperitoneal route 30 min before the forced swimming test or the open-field test.

The doses of drugs used were selected on the basis of experiments previously performed in our laboratory and on the literature data previously reported as not affecting locomotor activity (Jesse et al., 2010; O'Neill and Conway, 2001; Tolardo et al., 2010; Zomkowski et al., 2004).

2.4. Forced swimming test (FST)

The test was conducted using the method of Porsolt et al. (1977) with some modifications. Mice were individually forced to swim in an open plexiglass cylinder (diameter 10 cm, height 25 cm), containing 19 cm of water at 25 ± 1 °C, and the total immobility time during a 6 min test was scored live (Brocardo et al., 2008; Rosa et al., 2008; Tolardo et al., 2010; Zomkowski et al., 2004). As reported, classical antidepressants decreased immobility time in this paradigm (Brocardo et al., 2008; Dhir and Kulkarni, 2007; Kaster et al., 2007; Rosa et al., 2008; Yamada et al., 2004). Each animal was judged to be immobile when it stopped struggling and remained floating or motionless in the water, making only the movements necessary to keep its head above water. The absence of hind leg movement was recorded as immobility by stopwatch accumulation by a single observer during the exposures.

2.5. Mechanisms involved in the antidepressant-like effect of H. brasiliense extract and podoandin

To address some of the mechanisms by which *H. brasiliense* extract or podoandin causes antidepressant-like action in the forced swimming test, animals were pretreated with different pharmacological agents.

In order to investigate the possible contribution of the serotonergic system to the anti-immobility effect of *H. brasiliense* extract or podoandin in the forced swimming test, animals were pretreated with PCPA (100 mg/kg, a serotonin synthesis inhibitor) or saline, once a day, for 4 consecutive days. In previous studies, this treatment regimen of PCPA produced around 60–90% depletion of brain serotonin concentration in rats (Redrobe et al., 1998; Wang et al., 2008). The animals received treatment with *H. brasiliense* extract (50 mg/kg, i.p.), podoandin (10 mg/kg, i.p.) or vehicle 24 h after the last PCPA or saline injection, and were tested in the forced swimming test 30 min later. In separate experiments, animals were pretreated with ondansetron (a $5HT_3$ selective receptor antagonist, 0.3 mg/kg), NAN 190 a 5-$HT1_A$ selective receptor antagonist (0.5 mg/kg, i.p.), ketanserin a 5-$HT2_A$ receptor antagonist, (5 mg/kg, i.p.), pindolol a 5-$HT1_A/1_B$ receptor antagonist (10 mg/kg, i.p.) or vehicle, 15 min before the